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Anticancer Activity and DNA Fragmentation of Ferulic Acid from Banana peel using HeLa cell line.

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ABSTRACT: ***Ferulic acid is a phenolic ubiquitous plant antioxidant. The major focus of the present work was to study isolation of ferulic acid from banana peel using Staphylococcus aureus. The biosynthesized ferulic acid was identified and quantified by HPLC and TLC technique. It was confirmed about 179mg of ferulic acid was obtained from 60g of banana peel after 7th day of incubation period. The comparison between synthetic ferulic acid and biosynthesized ferulic acid were used to treat cervical cancer cell growth in a dose dependent manner. Both synthetic and biosynthesized ferulic acid are treated with HeLa cell line which exhibited increase in DNA fragmentation. The present research addresses ferulic acid can be biosynthesized by using fruits and vegetables waste into a valuable compound and to avoid toxic effect.***

KEYWORDS: ***Ferulic acid, Staphylococcus aureus, Banana peel, HeLa cell, DNA Fragmentation.***

INTRODUCTION

Cervical cancer is a type of cancer affects women. The vast majority of cervical cancer is caused by infection with certain subtypes of human papilloma virus (HPV), a sexually transmitted virus that infects cells and may result in precancerous lesion and invasive cancer [1]. Ferulic acid is phytoconstituent which exhibited diverse pharmacological effects including anticancer and pro-apoptotic properties [2,3]. Ferulic acid is a hydroxycinnamic acid. It is a type of organic compound. It is an abundant phenolic acid. It is related to trans-cinnamic acid where present in the plant kingdom and occurs mainly in the cell wall of cereal plants, seeds; fruit peels which is covalently linked to lignin with ether bonds [3]. Ferulic acid contains many physiological functions including antioxidant [5] antimicrobial, anti-inflammatory, anti-thrombosis, activities and also has antibiotic properties [6]. Antioxidant activity of ferulic acid able to competitively inhibit HMG-CoA reductase and activate glucokinase, contributing to reduce hypercholesterolemia and hyperglycemia. Ferulic acid inhibited the viability of various cancer cells. The present study was designed to investigate the cytotoxic and apoptotic potential of ferulic acid in HeLa cell lines by assessing the cell viability, antioxidant levels and DNA fragmentation.

MATERIALS AND METHODS

Preparation of ferulic acid from banana peels:

Banana peel was collected from nearby market Lifeteck Research Centre, Chennai, Tamil nadu. The banana peels were processed for the production of ferulic acid. The banana peels were cut into small pieces and cleansed with 100ml of 1.5% of Tween 20 solution along with water. The wasted banana peels were autoclaved for 20 minutes at 121oC (inactive any endogenous enzyme and proteinaceous) and dried in hot air oven. The dried banana peels was used as carbon sources for production of ferulic acid.

Release of ferulic acid:

Pure culture of Staphylococcus aureus strain was obtained from Lifeteck Research Centre R&D division, Chennai, Tamilnadu. The microorganism was grown in minimal medium [7] containing banana peels as a carbon source. The initial pH of the minimal medium was adjusted to 7.0 before autoclaving for 15 min at 121oC. The cultures were incubated at 35oC and analyses were carried out on day basis up to 10 days of incubation.

Analysis of ferulic acid:

Culture supernatants were prepared by filtration process and were acidified (pH 1-2) and extracted with equal volume of ethyl acetate. The ethyl acetate was evaporated using the distillation process. The sample was stored at -20oC.The residue was dissolved in 50% methanol and applied to thin layer chromatography by comparing with synthetic ferulic acid. Synthetic ferulic acid was obtained from was purchased from Sigma-Aldrich Chemical Pvt. Ltd., Bangalore, India.

Identification of ferulic acid:

Identification of ferulic acid was carried out by HPLC method. The identification of ferulic acid was confirmed by comparing retention times of synthetic ferulic acid.

Antioxidants activities

•**DPPH Assay:** DPPH (1,1-diphenyl-2picrylhydrazyl) is characterized as a stable free radical by virtue of the delocalization. 3.7ml of absolute methanol was added to sample test tube which contain 0.1ml of sample and 3.8ml of absolute methanol was added to standard test tube which contain 0.1ml of synthetic ferulic acid. Then 0.2ml of DPPH reagent was added to sample test tube and standard test tube. Incubate at room temperature in dark condition for 30 minutes and the absorbance was read at 517nm [8].

•**Nitric Oxide:** Sodium nitroprusside in aqueous solution at physiological pH spontaneously produce nitrite oxide which interacts with oxygen to produce nitrite ions free radical. 0.1ml of sample, 0.9 ml of distilled water and 1ml sodium nitroprusside were added. Control experiment without the test compounds but with equivalent amount of solution was conducted in an identical manner. After 3 hours incubated samples were diluted with 1.0 ml of Griess reagents (N-(1-napthyl) ethylenediamine dihydrochloride and sulfanilamide). The absorbance of the pink color developed was observed at 550nm on spectrophotometer. Same procedure was done with standard used as synthetic ferulic acid [9].

Anti cancer activity:

MTT Assay:

The anticancer activity of biosynthesized ferulic acid was studied against HeLa cell Line. HeLa cell line was obtained from National Centre for Cell Science (NCCS), Pune, India. The cells were maintained in DMEM (Dulbecco's Modified Eagle Medium) with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO2 at 37 °C. Cells were grown in 24-well plates and incubated in 37oC with 5% CO2 condition. Once the cell reaches the confluence, the sample ferulic acid and synthetic ferulic acid (standard) were added and incubated for 24hrs. After incubation, the sample and standard were removed from the well and washed with phosphate-buffered saline (pH 7.4). 100µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) was added and incubated for 4 hours. After that 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically [10]. DNA Fragmentation: This method involves the isolation of apoptotic DNA fragment by lysing the isolated nuclei of apoptotic cells with NP-40 buffer. The apoptotic DNA fragments were released into the supernatant was separated by 1% agarose gel electrophoresis [11]. The harvested HeLa cells were pelleted by centrifugation and the pelleted cells were then treated for 10 seconds with lysis buffer. After lysis, then centrifugation was carried out for 5 minutes at 16000 g. The supernatant was collected and the extraction is repeated with same amount of lysis buffers. The collected supernatants were mixed with 1% SDS and treated with RNase A at 56oC, followed by digestion with proteinase K for 2 hr at 37ºC. The DNA was then precipitated with ethanol immediately after treating with ammonium acetate. The precipitate was dissolved in gel loading buffer and separated by electrophoresis in 1% agarose gels.

RESULTS AND DISCUSSION

Ferulic acid was extracted from banana peels using Staphylococcus aureus by solvent extraction method and it was confirmed by TLC and HPLC techniques. Further, the antioxidant studies are evaluated by DPPH and nitric oxide assay. Finally the anticancer activity was estimated based on IC50 values using MTT assay by treating the standard and sample against HeLa cells and DNA fragmentation.

• **TLC (Thin Layered Chromatography):** It was performed to identify and separated compounds based on retention factor.

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S.No.	Sample	Optical Density 517nm			DPPH activity (%)
		I	II	Average	
1	Control	0.819	_	0.819	_
2	Standard	0.080	0.082	0.081	90.10
3	Sample	0.206	0.208	0.207	74.72

The bands of the extracted ferulic acid were compared with the bands of the standard (synthetic ferulic acid). The bands are visualized by UV light. Biosynthesized ferulic acid was identified by using Rf values.

RF = Distance moved by the solute (b)

Distance moved by the solvent (a)

The standard and sample ferulic acid has RF value 0.9687 and 0.9375. Hence the results showed that the sample is found to have ferulic acid [12]

• **HPLC (High Performance Liquid Chromatography):** It is system consists of compact LC and CE driver for preparation of sample and it was controlled by EZ chrome elite software. The analysis of Phenolic acids is detected by HPLC detector system. The analysis of HPLC chromatogram was based on the retention time of both standard and sample ferulic acid was represented in Figure1&2 [13].

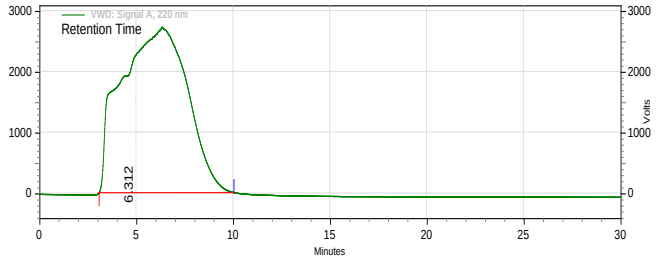

Figure1. HPLC chromatogram of Standard ferulic acid at 220 nm (Rt = 6.312).

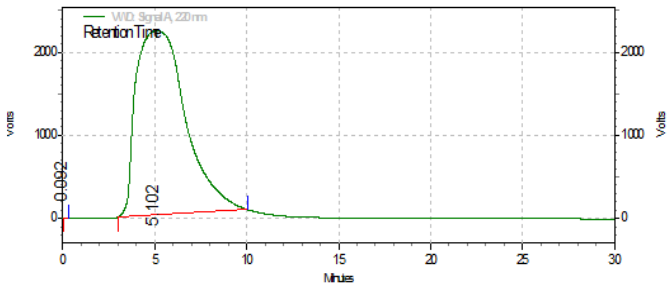

Figure2. HPLC chromatogram of Sample ferulic acid at 220 nm (Rt= 5.102).

The intense peak value for the sample is compared with standard (synthetic ferulic acid) and shows that the presence of ferulic acid in the sample.

•DPPH (1, 1-diphenyl-2-picrylhydrazyl) Assay: The antioxidant activity of standard and biosynthesized ferulic acid was calculated using DPPH Assay to delocalize the stable free radical. The antioxidant potential was expressed in Table 1.

Table 1-DPPH ASSAY

The antioxidant activity was determined by the formula

$$\% \text{ Antioxidant Activity} = \frac{\text{OD of control} - \text{OD of Test}}{\text{OD of control}} \times 100$$

• **Nitric Oxide Assay:** The antioxidant activity of standard and biosynthesized ferulic acid was calculated using Nitric oxide Assay to delocalize the stable free radical. The antioxidant potential was expressed in Table 2.

Table 2- Nitric Oxide Assay

S.No.	Sample	Optical Density 550 nm			NO_2 activity %
		I	II	Average	
1	Control	1.655	_	1.655	_
2	Standard	0.067	_	0.067	95.90
3	Sample	0.400	0.402	0.401	75.77

The antioxidant activity was determined by the formula

$$\% \text{ Antioxidant Activity} = \frac{\text{OD of control} - \text{OD of Test}}{\text{OD of control}} \times 100$$

The antioxidant activity of ferulic acid was calculated by DPPH and Nitric Oxide assays. It was expressed in percentage of DPPH molecules reduced by one molecule of the reducants. DPPH and Nitric oxide was characterizing the stable free radical by decolorization and it was estimated for standard and extracted ferulic acid from banana peels was obtained 74.72% and standard was obtained 90.10% in DPPH assay. In nitric oxide 75.77% for sample and for standard 95.90 % respectively. Hence results show that the sample ferulic acid is found to have antioxidant property [14, 15].

Anticancer Activity using MTT assay: The effect of synthetic and sample ferulic acid on cell viability by MTT assay and morphological changes in HeLa cells for 24 hr are depicted in Fig 3 and Fig 4.

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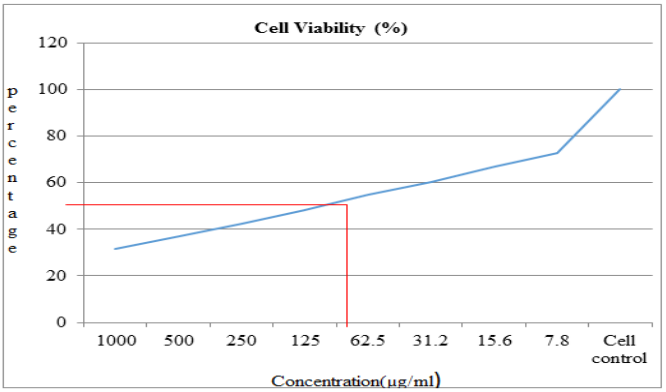

Figure 3- Anticancer activity effect of Synthetic ferulic acid on Hela cell line

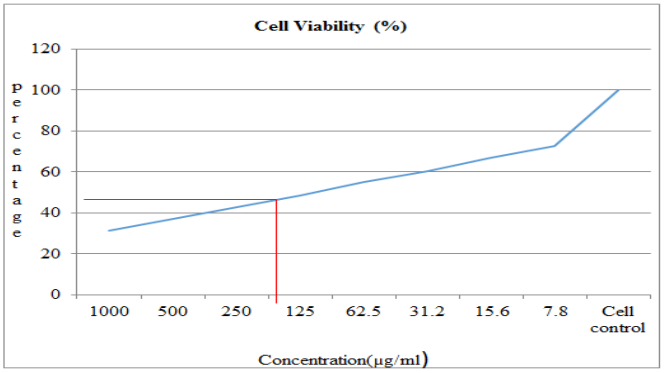

Figure 4 - Anticancer activity effect of Sample Ferulic acid on HeLa cell line.

The graph was plotted by using cell viability on the Y- axis and concentration of the sample on the X-axis. From graph shows the optimum cell viability of sample is 125µg/ml concentration and standard optimum cell viability is 62.5µg/ml concentration. Hence the results showed that the sample ferulic acid act as a anticancer drug [10].

DNA Fragmentation: DNA fragmentation in control and ferulic acid treated Hep-2 cells by their DNA ladder are shown in Fig. 5. We noticed significant increase in DNA fragmentation in HeLa cells treated with synthetic and biosynthesized ferulic acid for 24 hrs. DNA fragmentation assay using agarose gel electrophoresis could also help to evaluate the apoptotic potential of the test compound. Agarose gel electrophoresis revealed a typical DNA ladder for sample ferulic acid treated HeLa cells which showed more DNA fragmentation and further proves the cytotoxic or apoptotic potential of sample ferulic acid.

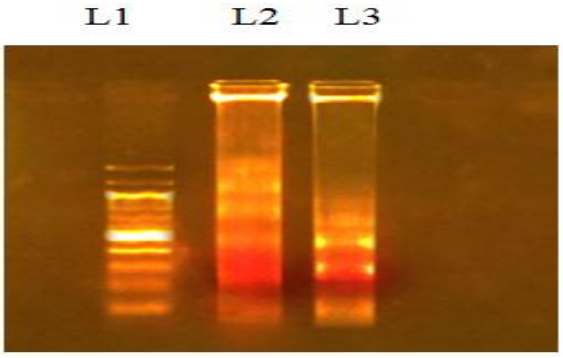

Figure 4- DNA Fragmentation

(L1 –DNA marker, L2- Synthetic ferulic acid.

L3- Sample ferulic acid.)

DNA fragmentation for the sample is compared with standard ferulic acid and the results shows the sample ferulic acid contain apoptotic property [11].

CONCLUSION

Ferulic acid produced from banana peels by using Staphylococcus aureus. The present study was carried out in the comparison of synthetic ferulic acid and ferulic acid extracted from banana peels to evaluate the antioxidant activity, anticancer activity and DNA fragmentation. It explores the cytotoxic potential of ferulic acid in HeLa cells. Based on the findings it is concluded that synthesized ferulic acid from banana peels can be used for cancer treatment.

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